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GLUCOSE AND SODIUM FLUXES ACROSS THE BRUSH BORDER OF *HYMENOLEPIS DIMINUTA* (CESTODA)[1]

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Tapeworms are a favorable material for studying the transport of organic solutes across membranes. The outer surface of adult tapeworms is comprised of a coenocytic epithelium with a highly developed brush border (Lumsden, 1966). Since tapeworms have no digestive tract, nutrients from the external medium must cross this external tegument. In addition, the intact tapeworms can be easily and rapidly maniupulated, making them ideal subjects for the study of transport kinetics.

Previous studies have shown that glucose transport in several species of tapeworms is Na^+-dependent (von Brand and Gibbs, 1966; Fisher and Read, 1971; Dike and Read, 1971a; Pappas and Read, 1972a; Pappas, Uglem and Read, 1973a). The present study was undertaken to characterize in some detail the relationship of glucose and sodium fluxes in the rat tapeworm, *Hymenolepis diminuta*.

Materials and Methods

Hymenolepis diminuta was maintained in the beetle, *Tenebrio molitor*, and male Sprague Dawley rats (Holtzman Co.) Rats weighing 60–80 g were infected with 30 cysticerocoids (*H. diminuta*) and worms were recovered 10 days post-infection. Worms from several rats were pooled and randomized into groups of 5, and preincubated for 15 min at 37 C in 15 ml of Krebs-Ringer saline containing 25 mM tris(hydroxymethyl)aminomethane-maleate buffer at pH 7.4 (KRT of Read, Rothman and Simmons, 1963), or in KRT with the NaCl replaced isomotically with KCl, LiCl, choline-Cl, or tris(hydroxymethyl)aminomethane-Cl. Other modifications of salines are described in the text. After preincubation worms were blotted on filter paper, placed in 5 ml of KRT (or the appropriate ion-substituted saline) containing radioactive substrate, and incubated for 2 min at 37 C. To terminate incubation each worm group was removed and rapidly rinsed in KRT, blotted on filter paper, and extracted overnight in 2 ml of 70% ethanol. Radioactivity in aliquots of the ethanol extracts and incubation media was determined using a gas-flow counter or liquid scintillation spectrometer; influx rates were calculated by comparing the radioactivity extracted from worms with the specific activity of the incubation media. The ethanol extracted worms were dried overnight at 95 C and weighed. Uniformly labeled ^{14}C-D-glucose and $^{22}Na^+$ (as NaCl) were obtained from Amersham/Searle Corp.

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Results

Dike and Read (1971a) showed that the influx of glucose across the brush border of *H. diminuta* is sensitive to Na^+ in the incubation medium. Our preliminary experiments indicated that preincubation of worms in media without Na^+ also affects glucose influx. To examine this effect further, worms were preincubated for varying time periods in Na^+-free KRT with tris as the replacement

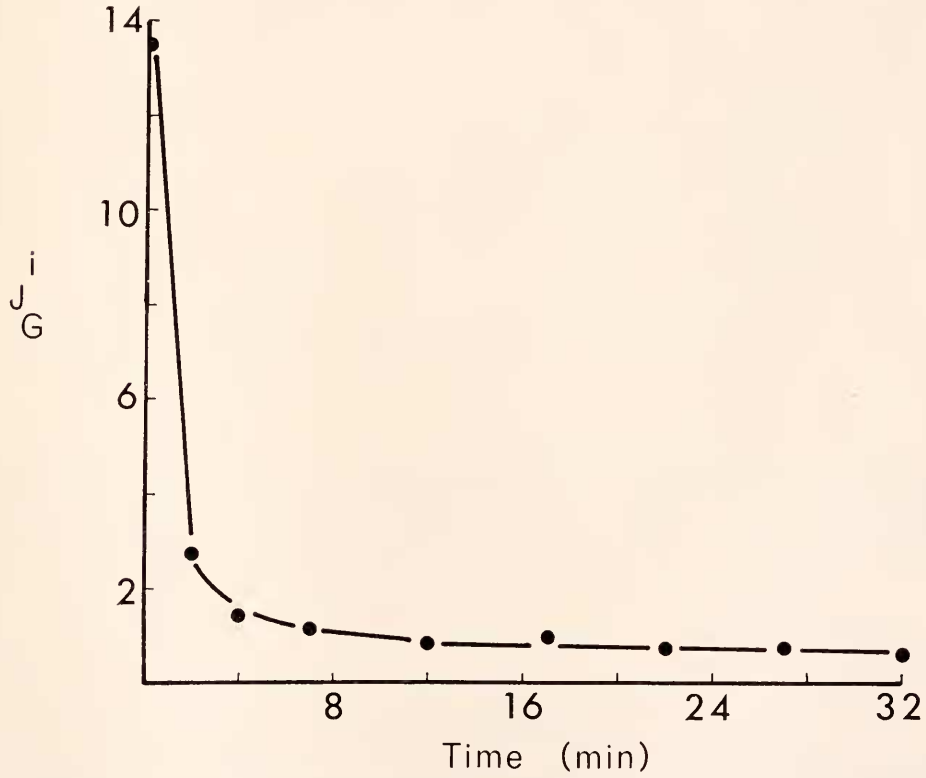

FIGURE 1. The effect of perincubation time in Na^+-free KRT on glucose influx (J^i_G = μmoles glucose absorbed/g ethanol extracted dry wt/2 min) in *Hymenolepis diminuta*. Worms were preincubated in Na^+-free KRT for predetermined time periods (abscissa), and then transferred to Na^+-free media containing 1 mM ^{14}C-glucose for 2 min. In this experiment, Na^+ was replaced with tris. Each point is the mean of 3 replicates.

cation, and then incubated for 2 min in Na^+-free KRT containing 1 mM ^{14}C-glucose. The results shown in Figure 1 demonstrated that a 12 min preincubation of worms in Na^+-free KRT was sufficient to inhibit glucose influx (J^i_G) 96%.

To determine the reversibility of the effect of Na^+ deletion on glucose influx, worms were preincubated in Na^+-free media for 30 min, incubated in KRT for varying time periods, and subsequently incubated for 2 min in KRT containing 1 mM ^{14}C-glucose. The results shown in Figure 2 demonstrated complete reversal of the effect of Na^+ deletion. In all subsequent experiments, worms were pre-

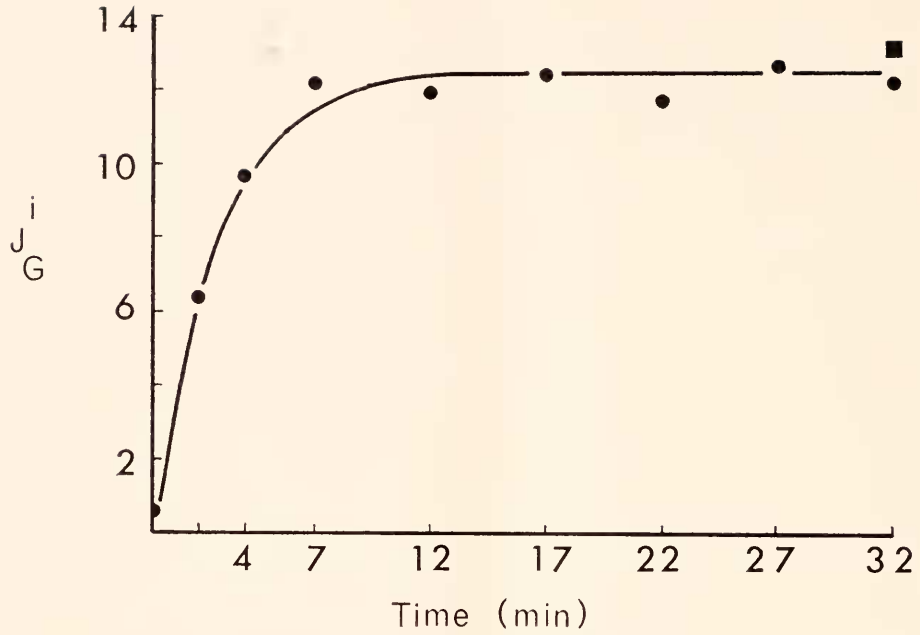

FIGURE 2. Reversal of the effects of Na^+ deletion on the influx of glucose (J^i_G, as in Fig. 1) in *Hymenolepis diminuta*. Worms were preincubated in Na^+-free KRT (tris as the replacement cation) for 30 min, followed by incubation in KRT ($[Na^+] = 154$ meq/l) for predetermined time periods (abscissa), and subsequently incubated in 1 mM ^{14}C-glucose in KRT for 2 min. The single square point represents glucose influx in a control group which was not incubated in Na^+-free KRT. Each point is the mean of 3 replicates.

incubated for 15 min in media containing the same cation concentrations in the final incubation medium.

It seemed desirable to examine the effects of various other cations as replacements for Na^+ in the medium. The effect on glucose influx of totally replacing Na^+ with Li^+, K^+, choline, or tris is shown in Table I. The much lowered influxes in media where K^+, tris, or choline were used as replacements for Na^+ were similar. However, glucose influx with Li^+ as the replacement cation was significantly higher ($P < 0.05$ by Student's t test) than influxes obtained with the other substituting

TABLE I

A comparison of 0.5 mM glucose influx (J_G^i = μmoles glucose absorbed/g ethanol extracted dry wt/2 min) in Hymenolepis diminuta *in salines with the Na^+ totally replaced with various cations and KRT. Each value is the mean ± S.E. of 3 replicates*

Replacement Cation	J_G^i	J_G^i in KRT
K^+	0.086 ± 0.005	5.66 ± 0.32
Choline	0.076 ± 0.006	4.96 ± 0.28
Tris	0.031 ± 0.004	5.28 ± 0.31
Li^+	0.56 ± 0.03	4.58 ± 0.22

cations, suggesting that Li^+ may partially replace Na^+. This "replacement" effect disappeared when increasing amounts of Na^+ were added to media containing Li^+. Glucose influx was a hyperbolic function of Na^+ concentration regardless of the replacement cation (Fig. 3).

Although the data did not indicate that K^+ competes with Na^+ in glucose influx, this possibility was examined further by determining the effects of K^+ concentrations on glucose influx at a constant glucose concentration (0.5 mM) and a suboptimal, fixed Na^+ concentration (25 meq/l) (isosmolarity was maintained by add-

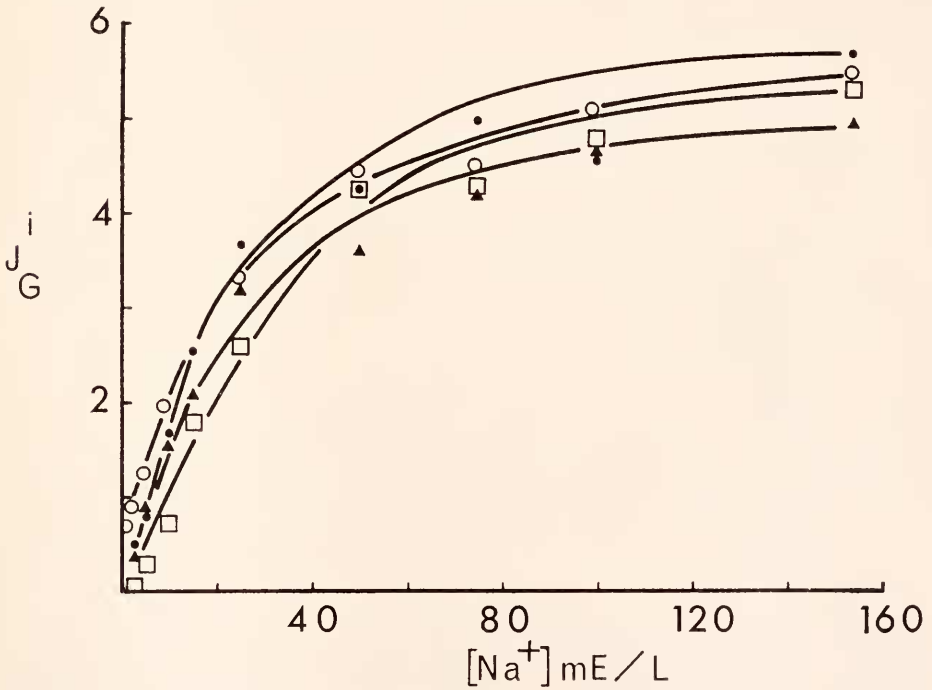

FIGURE 3. The influx of 0.5 mM ^{14}C-glucose (J^i_G as in Fig. 1) in *Hymenolepis diminuta* as a function of the Na^+ concentration ($[Na^+]$) in media with the Na^+ replaced with various cations. Replacement cations were K^+ (solid circles), Li^+ (open circles), tris (open squares), or choline (solid triangles). Each point is the mean of 3 replicates.

ing an appropriate amount of tris-Cl). There was no effect of K^+ on glucose influx at K^+ concentrations ranging from 0 to 100 meq/l.

Glucose influx as a function of glucose concentration followed apparent Michaelis-Menten kinetics in media with fixed Na^+ concentrations of 154, 50, 25, or 10 meq/l (Fig. 4). The apparent transport constant (K_t) for glucose influx was affected only slightly with decreasing Na^+ concentrations, but there was a marked decrease in the maximal influx of glucose ($J^i_G{}^{max}$) with decreasing Na^+ concentration (Table II).

When glucose influx was tested as a function of Na^+ concentration at several fixed glucose concentrations, it was apparent that glucose influx was a hyperbolic

function of Na^+ concentration, regardless of the glucose concentration. The data also showed that the Na^+ concentration necessary to achieve $J^i_{G^{max}}/2$ changed significantly at different glucose concentrations (Fig. 5, Table II). The kinetic parameters describing these glucose-Na^+ interactions are summarized in Table II.

While the above experiments demonstrated the dependence of glucose influx on Na^+, and suggested coupling of glucose and Na^+ fluxes, more direct evidence

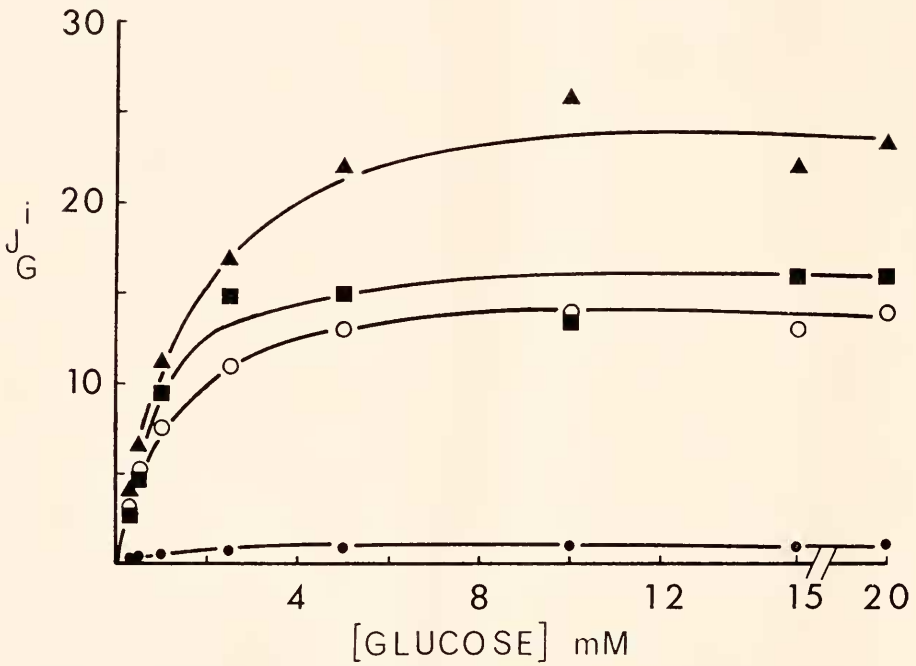

FIGURE 4. The influx of ^{14}C-glucose (J^i_G as in Fig. 1) in *Hymenolepis diminuta* as a function of glucose concentration in media with different fixed Na^+ concentrations. Na^+ concentrations of 154 meq/l (solid triangles), 50 meq/l (solid squares), 25 meq/l (open circles), and 10 meq/l (solid circles) were used. In all experiments, deleted Na^+ was replaced with tris. Each point is the mean of 3 replicates.

of coupling required a demonstration that Na^+ influx (J^i_{Na}) was related to the presence of glucose in the external medium. As shown in Table III, a very large Na^+ influx was associated with glucose influx.

Before carrying out experiments designed to evaluate coupling coefficients (J^i_{Na}/J^i_G), it was necessary to determine that the influxes of ^{14}C-glucose and $^{22}Na^+$ followed first order kinetics for the time period employed. Groups of worms were incubated in solutions containing either 25 meq/l $^{22}Na^+$ and 5 mM glucose, or 25 meq/l Na^+ and 5 mM ^{14}C-glucose. Samples were removed at 15 sec intervals, washed for approximately 2 sec in KRT and extracted in 70% ethanol. The influxes of Na^+ and glucose were a linear function of time for at least 2 min (Fig. 6).

To determine coupling cofficients, using labeled Na^+ and labeled glucose, incubations were conducted for 2 min with less than a 2 sec postincubation rinse in KRT.

The results of such an experiment are shown in Figure 7. In this experiment groups of worms were incubated in KRT containing 25 meq/l $^{22}Na^+$ and varying glucose concentrations, or 25 meq/l Na^+ with varying concentrations of ^{14}C-glucose. The plot of Na^+ influx versus glucose influx has a slope of 1.6 by regression analysis, indicating the coupling coefficient was at least 2. The intercept of the line in Figure 7 represents Na^+ influx not associated with glucose influx. As expected, the glucose-coupled Na^+ influx was a hyperbolic function of glucose concentration (Fig. 8). It should be noted that the concentration of Na^+ yielding a half-maximum rate of Na^+ influx in the presence of 5 mM glucose was about 154 meq/l. In a different type of experiment, the glucose concentration was held constant and

TABLE II

A summary of the kinetic parameters describing ^{14}C-glucose influx in Hymenolepis diminuta *as functions of Na^+ and glucose concentrations (meq/l and mM, respectively). The graphic analysis of V vs. $V/[S]$ (Dixon and Webb, 1964), with calculated regression lines, was used to determine these parameters*

Summary of Figure 4

$[Na^+]$	$J_G{}^{i\ max}$*	K_t**
154	26.3	1.4
50	17.1	1.0
25	14.7	0.9
10	1.1	0.8

Summary of Figure 5

[Glucose]	$J_G{}^{i\ max}$	K_t†
5	28.0	56
1	10.1	43
0.5	6.8	40
0.1	1.7	34

* maximal glucose influx = μmoles absorbed/g ethanol extracted dry wt/2 min.
** that [glucose] necessary to achieve $\frac{1}{2}\ J_G{}^{i\ max}$.
† that $[Na^+]$ necessary to achieve $\frac{1}{2}\ J_G{}^{i\ max}$.

the Na^+ concentration varied. Results of such an experiment are presented in Table IV. When $^{22}Na^+$ influx in the presence of glucose was corrected for the amount of Na^+ entering the worms in the absence of glucose, the apparent coupling coefficient varied as an inverse function of the Na^+ concentration. That is, with decreasing Na^+ concentrations the coupling coefficient increased. Thus, the coupling coefficient appeared independent of the glucose concentration over a 50-fold range, but inversely dependent on the Na^+ concentration over a 20-fold range.

When $^{22}Na^+$ influx was measured as a function of Na^+ concentration in the absence of glucose, it was found to be a nonlinear function of Na^+ concentration. This suggested that $^{22}Na^+$ influx in the absence of glucose did not occur by simple diffusion. This was examined further by adding varying quantities of unlabeled Na^+ in the presence of a fixed quantity of $^{22}Na^+$. If Na^+ influx was by diffusion alone, the addition of unlabeled Na^+ should not have affected the influx of $^{22}Na^+$.

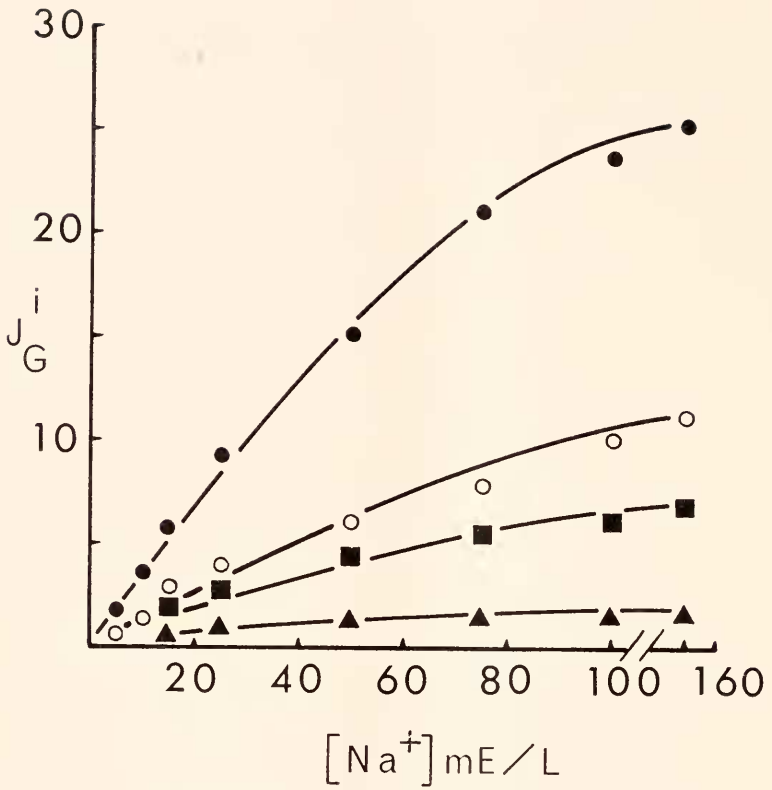

FIGURE 5. Influx of ^{14}C-glucose (J^1_G as in Fig. 1) in *Hymenolepis diminuta* as a function of Na^+ concentration in media with different glucose concentrations. Glucose concentrations of 5 mM (solid circles), 1 mM (open circles), 0.5 mM (solid squares), and 0.1 mM (solid triangles) were used. In all experiments, deleted Na^+ was replaced with tris. Each point is the mean of three replicates.

Such was not the case (Fig. 9). The data indicated that in the absence of glucose about 60% of the $^{22}Na^+$ influx at this concentration (10 meq/l) occurred by a mediated process, and about 40% by diffusion.

TABLE III

Influx of $^{22}Na^+$ ($J_{Na}{}^i$ = μequivalents absorbed/g ethanol extracted dry wt/2 min) as a function of the Na^+ concentration ([Na^+], meq/l) in Hymenolepis diminuta *in the presence or absence of 5 mM glucose. Each value is the mean ± S.E. of 3 replicates*

[Na^+]	$J_{Na}{}^i$ (with glucose)	$J_{Na}{}^i$ (without glucose)
5	3.07 ± 0.28	0.7 ± 0.1
15	8.78 ± 0.51	0.9 ± 0.1
25	14.33 ± 1.21	1.38 ± 0.01
50	26.33 ± 2.11	1.64 ± 0.02
100	36.52 ± 2.93	3.22 ± 0.02

In the absence of external Na^+, glucose influx in *H. diminuta* as a function of glucose concentration appeared saturable, with an approximate $J^i_G{}^{max}$ and K_t of 0.15 μmoles/g ethanol extracted dry wt/2 min and 0.5 mM, respectively. The inhibition of 0.1 mM ^{14}C-glucose influx by unlabeled glucose indicated that at least 80% of the glucose influx in Na^+-free media was mediated.

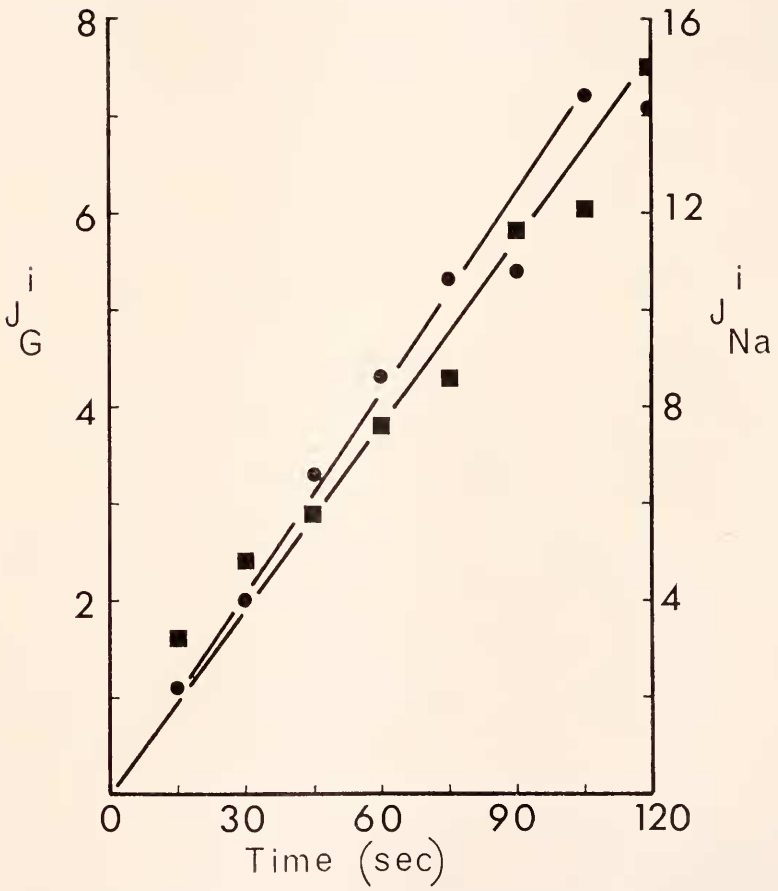

FIGURE 6. Fluxes of 5 mM ^{14}C-glucose (J^i_G = μmoles absorbed/g ethanol extracted dry wt, left ordinate) and 25 meq/l ^{22}Na (J^i_{Na} = μequivalents absorbed/g ethanol extracted dry wt, right ordinate) into *Hymenolepis diminuta* as a function of time; J^i_G = solid circles, J^i_{Na} = solid squares. Delected Na^+ was replaced with tris. Each point is the mean of 3 replicates.

The above observations suggested that further studies of the mediated influx of ^{14}C-glucose in the absence of Na^+, and of the mediated portion of $^{22}Na^+$ movement in the absence of glucose, were desirable. The glycoside phlorizin was a competitive inhibitor of ^{14}C-glucose influx in *H. diminuta* (Fig. 10). In the absence of external Na^+, phlorizin abolished over 70% of the residual ^{14}-C- glucose influx suggesting that the same glucose transport system was involved in the presence and absence of external Na^+. On the other hand, phlorizin had no effect on

the influx of $^{22}Na^+$ in the absence of glucose, suggesting the possibility of mediated Na^+ influx which was independent of the glucose transport system.

Discussion

The data of the present paper are consistent with the basic elements of Crane's (1962, 1965) Na^+-gradient hypothesis for the transport and accumulation of sugars (see also Schultz and Curran, 1970). Previous studies (Pappas, Uglem and Read, 1974) have shown that glucose is indeed accumulated by *H. diminuta,* and cotrans-

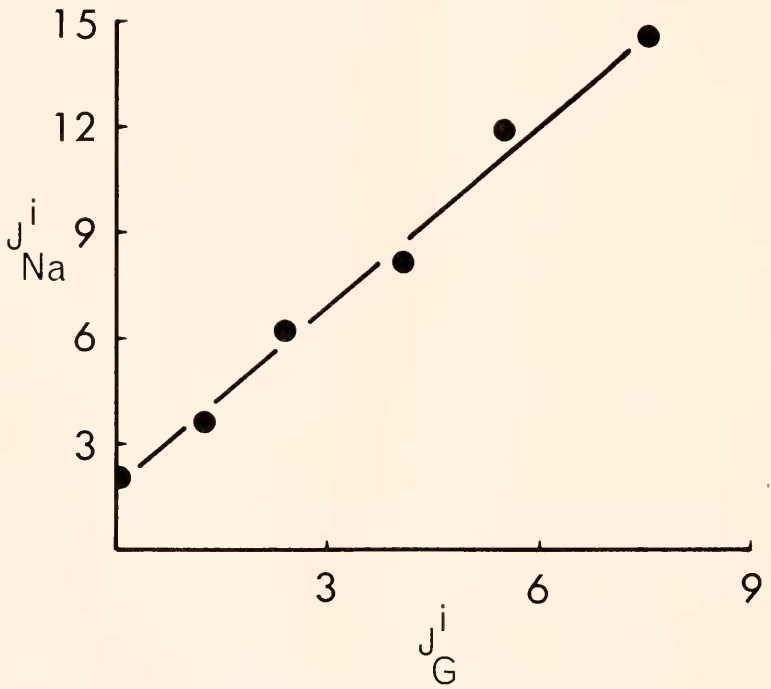

Figure 7. The relationship of ^{14}C-glucose influx (J^i_G as in Fig. 1) and $^{22}Na^+$ influx (J^i_{Na} = μequivalents absorbed/g ethanol extracted dry wt/2 min) in *Hymenolepis diminuta* when the Na^+ concentration was 25 meq/l, and the glucose concentration was 0, 0.1, 0.25, 0.5, 1 and 5 mM. Deleted Na^+ was replaced with tris. Each point is the mean of 3 replicates.

port of glucose and Na^+ across the brush border of *H. diminuta* is demonstrated unequivocally in the experiments described herein.

When Na^+ is deleted from the medium, K^+, choline, or tris do not substitute for Na^+ in enhancing glucose influx, while Li^+ appears to substitute to a small extent. The effects of Li^+ disappear with increasing Na^+ concentrations. Similar effects of Li^+ on glucose influx in the tapeworm *Calliobothrium verticillatum* were reported by Pappas and Read (1972a). Pappas *et al.* (1973a) found that K^+ competes with Na^+ in the glucose transport system of the larval tapeworm *Taenia crassiceps,* and a similar interaction between K^+ and Na^+ has been observed in the tetra-

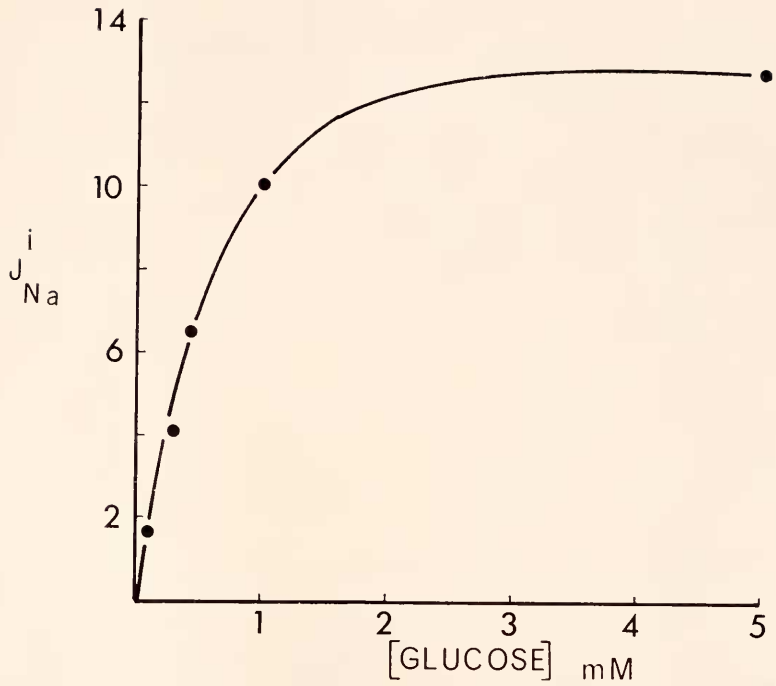

FIGURE 8. Influx of 25 meq/l $^{22}Na^+$ (J^{i}_{Na} as in Fig. 7) in *Hymenolepis diminuta* as a function of glucose concentration. All data were corrected for that Na^+ movement which occurs in the absence of glucose. Each point is the mean of 3 replicates, and deleted Na^+ was replaced with tris.

phyllidean *C. verticillatum* by Fisher and Read (1971) and Pappas and Read (1972a). Competition between Na^+ and K^+ in Na^+-dependent transport systems in mammalian tissues is suggested by the data of Nathans, Tapley and Ross (1960), Kipnis and Parrish (1965), and Crane (1965). In contrast to the above systems, K^+ does not appear to interact with the Na^+-coupled glucose transport system of *H. diminuta*. Further, there is no evidence that glucose transport in *H. diminuta* is inhibited in K^+-free media, as has been observed in some mammalian tissues (Riklis and Quastel, 1958; Bihler and Crane, 1962). When the external Na^+

TABLE IV

The coupling coefficient ($J_{Na}{}^{i}/J_{G}{}^{i}$) for Na^+-coupled glucose influx in Hymenolepis diminuta *as a function of Na^+ concentration of the external medium ([Na^+], meq/l). In each experiment the Na^+ concentration was held constant and the glucose concentration varied from 0.1 to 5 mM. Coupling coefficients are calculated slopes of plots of $J_{Na}{}^{i}$ vs. $J_{G}{}^{i}$, as in Figure 7*

N*	[Na^+]	Coupling coefficient
18	5	1.90
18	25	1.60
18	50	0.87

* number of determinations used in calculating each coupling coefficient.

concentration is greater than zero, glucose influx in *H. diminuta* conforms to apparent Michaelis-Menten kinetics, and a decreased external Na^+ concentration results in a lower maximal glucose influx, as well as a somewhat lower K_t. Thus, between 10 and 154 meq/l Na^+, $J^i_{G}{}^{max}$ changes about 25-fold, while K_t changes less than 2-fold. This difference indicates that high Na^+ concentrations decrease

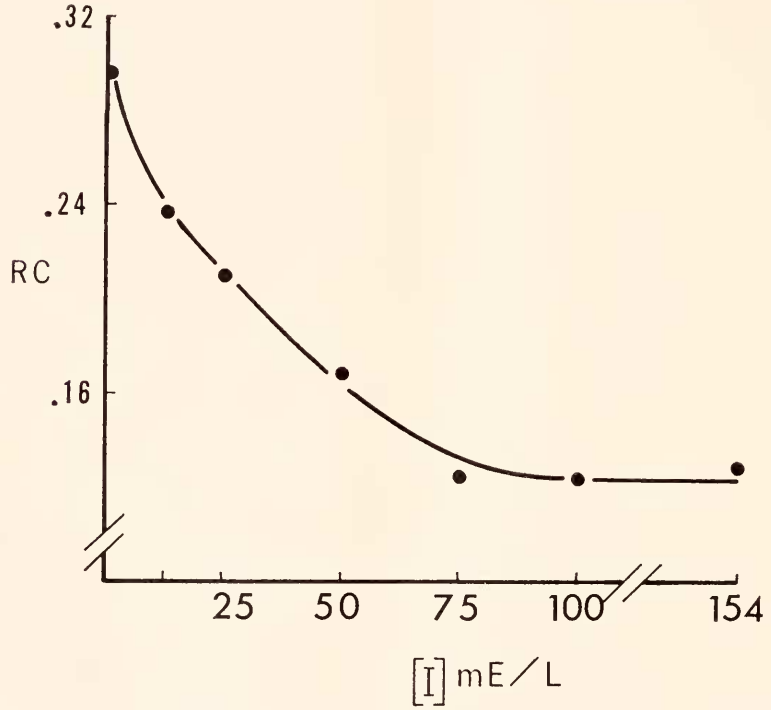

FIGURE 9. A plot of the rate constant (RC) for the influx of 10 meq/l $^{22}Na^+$ in *Hymenolepis diminuta* as a function of increasing concentrations of unlabeled Na^+ in the medium ([I]). Deleted Na^+ was replaced with tris, and appropriate amounts of tris were added such that $[Na^+] + [tris] = 154$ meq/l. The rate constant was calculated as follows: ([μequivalents of $^{22}Na^+$ absorbed by *H. diminuta*/ml worm water/2 min]/[μequivalents $^{22}Na^+$/ml incubation medium]). Thus, the units of the rate constrant are $(time)^{-1}$.

slightly the affinity of the carrier for glucose, but that the major effect of increasing Na^+ on glucose influx is an increased translocation rate of the complex formed by the carrier, glucose, and Na^+.

As previously reported by Dike and Read (1971a), the influx of glucose shows first order dependence on the Na^+ concentration of the medium. In this respect, *H. diminuta* resembles *C. verticillatum* (Pappas and Read, 1972a), and the mucosa of rabbit ileum (Goldner, Schultz and Curran, 1969). However, in *H. diminuta*, the coupling coefficient (J^i_{Na}/J^i_{G}) varies inversely with the Na^+ concentration, whereas in *C. verticillatum* and in the rabbit iluem the coupling coefficient is independent of the Na^+ concentration. In *C. verticillatum*, the coupling coefficient

is 2 or more, and in rabbit ileum it is unity. The coupling coefficients appear to be independent of the glucose concentration in all of these systems.

In showing first order dependence on Na^+ concentration with coupling coefficients greater than unity, glucose influx in *H. diminuta* resembles Na^+-coupled glucose influx in *C. verticillatum* (Pappas and Read, 1972a), and alanine influx in the pigeon erythrocyte (Wheeler and Christensen, 1967). In the latter system, Na^+-coupled influxes of asparagine, hydroxyproline, serine, and threonine have coupling coefficients of 2 or more, but show a first order dependence on the Na^+

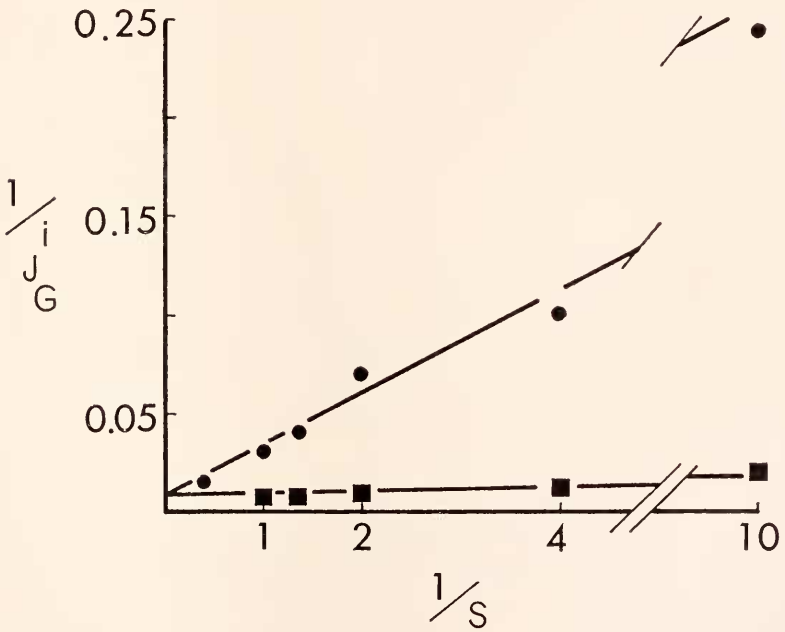

FIGURE 10. Effect of 0.05 mM phlorizin on the 2 min influx of ^{14}C-glucose (J^i_G as in Fig. 1) in *Hymenolepis diminuta;* S = glucose concentration, mM; influxes with phlorizin = solid circles; influxes without phlorizin = solid squares. Each point is the mean of 3 replicates, and lines were fitted by regression analysis.

concentration in the suspending medium (Koser and Christensen, 1968). On the other hand, *H. diminuta* differs from the above mentioned systems in that the coupling coefficient is an inverse function of the Na^+ concentration.

Any kinetic model which will describe the coupled influx of Na^+ and glucose across the brush border of *H. diminuta* must account for all of the properties of the *H. diminuta* system indicated above, particularly the first order dependence on Na^+ concentration with coupling coefficients exceeding unity. Such a model must include the following: (1) The binary complex of carrier and glucose, or binary or higher complexes of carrier and Na^+ (one or more Na^+), are translocated to a very minor extent. (2) Binding of one Na^+ to the carrier at a single specific site results in translocation of the glucose-carrier-Na^+ tertiary complex. (3) If the glucose-carrier complex is translocated at a very low rate, although free carrier is

freely translocated, there should be a tendency for pooling of this complex in the *cis* configuration (*i.e.*, at the external surface of the membrane) at very low Na^+ concentrations with glucose in the medium. (4) Further, binding of Na^+ apparently can occur in at least one non-effector site on the carrier, with only one site involved in effecting the translocation of the glucose-carrier-Na^+ complex; a change in the Na^+ concentration in the medium may change the coupling coefficient if the binding constants of the noneffector and effector Na^+ sites differ considerably.

Pappas and Read (1972a) observed coupling coefficients of at least 2 in the co-transport of glucose and Na^+ in *C. verticillatum*. It was postulated that there are 2 or more Na^+ binding sites on the glucose carrier, that there is no cooperative interaction between Na^+ binding sites, and that binding of Na^+ on any site will effect translocation of the glucose-carrier complex. However, *H. diminuta* differs from *C. verticillatum* in that the coupling coefficient changes as a function of Na^+ concentration, and the model postulated for *C. verticillatum* will not apply to *H. diminuta*.

With the above in mind, the following hypothesis will furnish a tentative explanation of the data: The glucose carrier has multiple sites for the binding of Na^+. Na^+ binding on at least one of these sites has negliglibe effector function in the translocation of glucose, and the non-effector site(s) is saturated at very low Na^+ concentrations. Translocation of Na^+ bound to non-effector sites is glucose-dependent, although the translocation of glucose is not dependent on Na^+ bound to non-effector sites. Binding of Na^+ at a single effector site acts in the translocation of glucose, and this effector site is not saturated at physiological concentations of Na^+. Thus, in the presence of glucose and low external Na^+ concentrations, binding and translocation of Na^+ on low affinity effector and high affinity non-effector sites would yield coupling coefficients of 2 or more. As the Na^+ increases to concentrations well above those which saturate the non-effector site, the Na^+ cotransported after combining with the non-saturated effector site becomes a larger and larger proportion of the cotransported Na^+, and the coupling coefficient decreases, approaching unity. This hypothesis may be amenable to experimental testing.

A small mediated influx of glucose occurs in the absence of Na^+ in the medium with *H. diminuta*, and Pappas and Read (1972a) found a small, but significant, mediated transport of glucose in the absence of Na^+ in *C. verticillatum*. However, in neither case can it be concluded that this involves a truly Na^+-free environment for the carrier. In *H. diminuta* there appears to be an unstirred layer adjacent to the glucose transport system which is not freely accessible to proteins (glucose oxidase) (Dike and Read, 1971a). Since Na^+ may well leak into this unstirred layer, the residual mediated transport of glucose, without Na^+ in the external medium, might involve the coupled transport of Na^+ and glucose, the latter of which has leaked into the unstirred layer from the worm's tissues. However, the failure of phlorizin to inhibit Na^+ influx in the absence of glucose argues against this latter hypothesis. It is of interest to note that in larvae of *T. crassiceps*, glucose influx in the absence of Na^+ occurs by diffusion (Pappas *et al.*, 1973a). Attempts to determine coupling coefficients for the influx of Na^+ and glucose in *T. crassiceps* have failed, although mediated glucose influx is Na^+-dependent. Influx of Na^+ in the absence of glucose is extremely high in *T. crassiceps* larvae, and coupled Na^+ influx may be masked (Pappas and Uglem, unpublished; Pappas *et al.*, 1973a).

On the other hand, measured coupling coefficients may be zero, though coupling occurs, if the rate constants for the processes involved are appropriate (Schultz and Curran, 1970). An elucidation of the nature of glucose and Na^+ movements in *T. crassiceps* larvae requires further experimentation.

Crane's (1965) data on the transport of sugar and Na^+ in the hamster intestine indicated that there is cooperative interaction between the binding sites. There is negligible cooperative interaction in binding of sugar and Na^+ in *H. diminuta*, and there is no evidence for such an interaction in *C. verticillatum* or rabbit mucosa (Pappas and Read, 1972a; Goldner *et al.*, 1969). In the two tapeworms and in rabbit mucosa, the main effect of Na^+ on glucose tansport seems to involve translocation of the glucose-carrier complex, rather than an effect of Na^+ on the affinity of the carrier for glucose.

H. diminuta shows a remarkable array of variations in dependence of transport on Na^+. Glucose transport is Na^+-dependent and Na^+-coupled. Glycerol transport is partially Na^+-dependent (Pittman and Fisher, 1972), and it has been shown that there are possibly two glycerol systems involved, one of which is Na^+-dependent and inhibited by 1,2-propanediol, and another which is insensitive to Na^+ and 1,2-propanediol. However, coupling of Na^+ and glycerol fluxes was not demonstratble (Uglem, Pappas and Read, 1974). In addition to *H. diminuta*, there is evidence for a relationship between Na^+ and sugar transport in three other tapeworms species, *Taenia taeniaeformis*, *T. crassiceps*, and *C. verticillatum* (von Brand and Gibbs, 1966; Fisher and Read, 1971; Pappas *et al.*, 1973a; Pappas and Read, 1972a). Contrary to the Na^+-dependence of glucose fluxes in *H. diminuta* and *T. crassiceps* larvae, amino acid fluxes and accumulation in these two tapeworms are apparently Na^+-insensitive (Read *et al.*, 1963; Pappas, Uglem and Read, 1973b, 1974).

The coupling of Na^+ and glucose fluxes in parasitic flatworms is of some interest from an evolutionary standpoint. It is generally agreed that flatworms constitute a primitive group of multicellular organisms. The occurrence of coupled Na^+ and sugar fluxes in such disparate systems as the hamster and rabbit brush borders and absorptive surfaces of tapeworms furnishes a strong argument that such systems are widely distributed in the animal kingdom, and that the evolution of such systems has been very conservative. Further, although it can be argued that obligately parasitic tapeworms have lost regulatory capacities in evolution (Read and Simmons, 1963), *H. diminuta* has retained a complex array of independent systems for regulating fluxes of organic solutes across the brush border. Specific transport systems in *H. diminuta* include at least four for amino acids (Read *et al.*, 1963), one for sugars (Phifer, 1960; Read, 1961), two for glycerol (Pittman and Fisher, 1972; Uglem *et al.*, 1974), at least three systems for purine and pyrimidine bases (MacInnis, Fisher and Read, 1965; MacInnis and Ridley, 1969; Pappas, Uglem and Read, 1973c), one for short-chain fatty acids (Arme and Read, 1968), one for long-chain fatty acids (Chappell, Arme and Read, 1969), and at least two for water-soluble vitamins (Pappas and Read, 1972b 1972c). In addition, *H. diminuta* possesses intrinsic tegumentary phosphohydrolases (Arme and Read, 1970; Dike and Read, 1971a, 1971b; Pappas and Read, 1974) which, in conjunction with the above transport systems, may play an important role in regulating organic fluxes across the tegument. Further, *H. diminuta* may also

alter the kinetic properties of host enzymes (Pappas and Read, 1972d, 1972e; Read, 1973; Ruff and Read, 1973).

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Summary

When *Hymenolepis diminuta* was preincubated in Na^+-free KRT (tris-maleate buffered Krebs-Ringer saline) for varying time intervals, followed by incubation in ^{14}C-glucose in Na^+-fee KRT, the influx of glucose in worms was lowered significantly. This effect of Na^+ deletion on glucose influx was totally reversible by incubating worms in KRT ($[Na^+] = 154$ meq/l).

When Na^+ in the medium was replaced with K^+, tris, or choline, a similar decrease in glucose influx in worms was noted; replacement of Na^+ with Li^+ resulted in a glucose influx rate significantly higher than that obtained with K^+, tris, or choline as the replacement cation. In media with a suboptimal Na^+ concentration (25 meq/l), influx of 0.5 mM glucose in worms was unaffected by varying concentrations of K^+ (0–100 meq/l).

Apparent Michaelis-Menten kinetics were observed when glucose influx as a function of glucose concentration was determined in media with Na^+ concentrations of 154, 50, 25, and 10 meq/l. There was a slight decrease in the apparent transport constant (K_t) for glucose influx and a marked decrease in maximal glucose influx ($J^i_G{}^{max}$) with decreasing Na^+ concentration. Glucose influx in *H. diminuta* as a function of the Na^+ concentration displayed apparent Michaelis-Menten kinetics, and the Na^+ concentration necessary to attain $J^i_G{}^{max}/2$ lowered significantly at lower glucose concentrations.

A large Na^+ influx was associated with glucose influx in *H. diminuta*, and the influxes of Na^+ and glucose exhibited first order kinetics for at least 2 min. In media with Na^+ concentrations of 5, 25, and 50 meq/l, coupling coefficients (J^i_{Na}/J^i_G) were 1.90, 1.60, and 0.87, respectively, or inversely related to the Na^+ concentration. Coupling coefficients were independent of the glucose concentration over a 50-fold range. Glucose-coupled Na^+ influx in worms was a hyperbolic function of glucose concentration. In the absence of glucose, Na^+ influx in *H. diminuta* apparently occurred, in part, by a mediated process which was unaffected by phlorizin. There was a small mediated glucose influx in worms in the absence of external Na^+, 70% of which was abolished by phlorizin.

The cotransport of Na^+ and glucose in *H. diminuta* is (1) compared with other similar systems in both tapeworms and mammals, (2) presented in support of Crane's Na^+-gradient hypothesis for transport and accumulation of sugars, (3) tentatively explained in the form of a hypothesized model, and (4) discussed briefly in relation to the evolution of parasitic flatworms.

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